

## PRRS RFLP, what is it and how we use them?

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Several different strategies have been used to classify the different PRRSV type-2 viruses into epidemiologically meaningful groups. In this science page, we discuss RFLPs (restriction fragment length polymorphisms), one of the most commonly used ways of classifying this virus, highlighting how it works and its limitations.

RFLP classification for PRRSV was originally proposed in 1998 as a way of differentiating wild (field) strains from vaccine strains (1). In this original description, three restriction enzymes (*MluI*, *HincII* and *SacII*) were used to fragment or cut the PRRSV genome. The **location** where each enzyme binds in the PRRSV ORF5 genome to make the cuts can be determined based upon the size of the fragments obtained in a gel electrophoresis run. The pattern of where the combination of these enzymes binds and cuts the PRRSV2 ORF5 genome were described as RFLP cut patterns. Today, the viral genome does not need to be exposed to enzymes or run-in gel electrophoresis. The RFLP of a genome can be determined by a computer program.

### How RFLPs are used today?

1. **Differentiate wild-type PRRS from vaccine strain viruses.**
2. **Initial characterization of a virus (i.e. during an outbreak).**
3. **In combination with another classification method called lineages to better identify an epidemiologically meaningful group of viruses.** The most recent example is the newly emerged Lineage 1C RFLP 144 variant (2). When first identified, this group was initially described as RFLP 1-4-4 viruses. However, only 33% of all currently circulating RFLP 1-4-4 viruses were associated with this outbreak because many genetically unrelated sequences share the same RFLP pattern. In contrast, only 42% of all currently circulating L1C viruses were associated with this outbreak because lineage classification is too broad to identify a specific subgroup of sequences. By combining both (lineage 1C and RFLP 1-4-4) we found that 81% of all currently circulating L1C 144 viruses were associated with this outbreak. It is still imperfect when compared to a phylogenetic approach, but the combination of a broad lineage category with a very granular RFLP classification might be more epidemiologically meaningful depending on the question being addressed.

### There are several caveats with RFLPs though, which need to be emphasized:

1. **Does not track genetic relationship**
  - a. RFLPs have been shown to change in as few as 10 animal passages (3). Theoretically, a single mutation (depending on where it occurs) has the potential to change the RFLP type. This is referred to as “RFLP instability”.
  - b. Potential for two distantly related viruses to share the same RFLP type (see Supplementary Table 1 of Paploski 2021) (4). With the recently emerged Lineage 1C RFLP 144 variant we witnessed a significant level of confusion as producers/veterinarians thought that farms located in the eastern corn belt that broke with a 144 virus had the same virus that was being found in Minnesota and Iowa. They were both different viruses. In other words, if two viruses share the same RFLP pattern this does not assure they are the same virus until sequences are compared.
2. **Immunological meaning of an RFLP is unclear:** It is important to remember that the RFLPs were originally proposed to distinguish between the one vaccine available at the time and wild-type strains. In other words, if a herd went through a 144 outbreak, it would not necessarily mean the herd will be fully protected against all 144 viruses.
3. **Potential for many different RFLP patterns:** more than 14 thousand patterns are currently possible (and the list increases with time).
4. **RFLP is ORF5 focused:** Two viruses can have similar ORF5 sequences, but the remaining sections of the genome can be different. If a test result came back as a RFLP 252 (the same pattern of a live-virus vaccine), it cannot be assumed that the whole virus is a vaccine-like strain since the other segments could be part of another virus as a result of a recombination.

In general, RFLP classification can help in answering some questions but should not be overinterpreted beyond the limitations of the classification. Often, more information are needed to reach a better and objective conclusion about the virus being investigated. Work still needs to be done to have a better and validated system for PRRSV 2 classification.

### References

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